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EFFECT OF AMINOPTERIN AND SEXUAL HORMONES IN THE TOAD

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FOLIC ACID antagonists inhibit the hypertrophic effect of estrogens on the oviduct of the chicken (Hertz, 1948; Franklin et al., 1948; Hertz and Tullner, 1949) and on the ovaries of the monkey (Hertz and Tullner, 1949). Inmediately after metamorphosis it was observed in the *Rana clamitans* that the oviduct growth produced by estradiol administration was increased by folic acid and decreased by aminopterin (Goldsmith and col., 1948 to 1950). Later, Velardo and Hisaw (1952-1953) observed that aminopterin prevents the response of the uterus in castrated rats injected with progesterone, as well as the decidual reaction in normal animals or in ovariectomized rats showing pseudopregnancy and having received progesterone.

These changes in the action of sexual hormones due to aminopterin administration led us to study the influence of the drug on the effect of testosterone propionate in prepuberal male toads, of estradiol in prepuberal female toads and of progesterone in adult female toads. All the modifications occurring in the weight of testes and the apparition of the thumb callosity were studied in the first named; the weight of the oviduct and the ovaries in the second and the variations in the weight of the oviduct in the third.

METHODS

Two hundred *Bufo arenarum* Hensel (100 males and 100 females) were used, of small size, sexually inmature, weighing from 10 to 30 grams. Males were divided in groups of 20, as follows: 1) controls sacrificed on the beginning day of the experiment; 2) controls sacrificed on the last day of the experiment, three weeks later; 3) injected with 0.1 mg of aminopterin (4-amino pteroylglutamic acid) in the dorsal lymphatic sac, three times a week; 4) injected with 1 mg of testosterone propionate twice a week; 5) injected with aminopterin and tes-

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tosterone, same dosis and frequency as lots 3 and 4. Female: 1) controls sacrificed on the beginninig day of the experiment; 2) controls sacrificed at the end of the experiment, three weeks later; 3) injected with 0.1 mg of aminopterin, three times a week; 4) injected with 0.5 mg estradiol benzoate, twice a week; 5) injected with aminopterin and estradiol benzoate, same dosis and frequency as lots 3 and 4. As toads were growing, each animal was fed approximately 2 g of fresh beef liver once a week in order to prevent any effect of prolonged fasting

TABLE I

Male toads weighing 10 to 30 g; each figure represents the arithmetic mean of 20 toads

Group	Body weight g		Weight of Testes mg		Number of toads with dark thumb callosity
	Initial	Final (after 21 days)	Initial	Final	
Initial controls	16.9 ± 2.2	—	10.1 ± 1.8	—	0/20
Final controls	17.3 ± 3.1	15.6 ± 2.7	—	10.5 ± 2.1	0/20
Aminopterin injection	19.2 ± 2.9	16.6 ± 1.7	—	10.5 ± 2.4	0/20
Testosterone propionate injection	19.7 ± 3.6	18.2 ± 2.5	—	21.0 ± 3.3	9/20
Aminopterin + Testosterone propionate	18.2 ± 1.7	17.3 ± 3.6	—	13.1 ± 1.9	3/20

*: ± standard error.

or excessive feeding on the results of the experiment. Initial and final body weights were recorded. After sacrificing the animals, in the males the weight of the testes and the presence or absence of thumb callosity and in the females the weight of the oviduct and the ovaries were determined.

For the third experiment, 12 adult female toads were used, divided in two groups of animals each. One group was injected in the dorsal lymphatic sac, with 1 mg of aminopterin in 2 ml Na Cl solution (0.8 %) every other day. Three weeks later the two groups were operated, removing and weighing the right oviduct and isolating, between two ligatures, a large part of the left side oviduct, following the technique described by Allende (1938). Shortly thereafter, 6 of them were injected for two days with 1 mg of progesterone (microcrystals) in NaCl solution (0.8 %). Forty-eight tours after the last injection, the animals

were sacrificed, and the left oviduct weighed. During all the experiments, the animals were maintained at a temperature of 23° C.

RESULTS

Table I presents the mean values obtained in prepuberal male toads. In all the groups, final body weight was lower than the initial.

TABLE II

Prepuberal female toads weighing 10 to 30 g; each figure represents the arithmetic mean of 20 toads

	Body weight g		Weight of the Oviduct mg		Weight of the Ovary mg	
	Initial	Final (after 21 days)	Initial	Final	Initial	Final
Initial controls	15.8 ± 2.2	—	1.4 ± 0.4	—	82.3 ± 7.1	—
Final controls	17.2 ± 3.2	15.9 ± 2.6	—	1.9 ± 0.6	—	96.1 ± 9.2
Aminopterin injection	19.8 ± 4.0	17.7 ± 3.1	—	1.6 ± 0.4	—	92.3 ± 11.2
Estradiol benzoate injection	19.2 ± 2.1	17.5 ± 1.8	—	182.1 ± 26.2	—	140.0 ± 16.6
Aminopterin + Estradiol benzoate	19.1 ± 1.6	17.5 ± 2.9	—	79.9 ± 19.3	—	120.6 ± 11.8

The final weight of testes was only increased in toads given testosterone propionate alone or associated with aminopterin which reduced but did not prevent the effect of testosterone. The thumb callosity in the anterior limbs of the toad is a secondary sexual character, which appears under the action of testosterone. Its frequency was decreased by aminopterin.

Table II shows the values obtained in prepuberal female toads. Body weights were decreased in all groups while the weight of the oviduct and ovary was increased by the action of estradiol benzoate. Aminopterin decreased the activity of this hormone in the doses employed, but it did not completely inhibit it.

The results of the third expermient are expressed in table III, demonstrating that aminopterin inhibits the secretory effect of progesterone on the oviduct.

DISCUSSION

Estradiol benzoate exerts a stimulating effect on the growth of

TABLE III

Aminopterin administration (1 mg) every other day, during 3 weeks. Later, progesterone (1 mg) during 2 days. All the toads were sacrificed 48 hours after the last injection

Group	Body weight g	Right oviduct Initial weight g	Left oviduct Final weight g	Difference %	$\frac{m_1 - m_2}{\sqrt{(\varepsilon_1)^2 + (\varepsilon_2)^2}}$
Controls	195 ± 4.8	4.722 ± 0.229	8.085 ± 0.368	+ 71.2	7.8
Aminopterin injection	193 ± 6.2	4.225 ± 0.219	5.090 ± 0.273	+ 20.4	2.5

the oviduct (Houssay, 1950; Galli-Mainini, 1950; Penhos, 1950). This effect is reinforced by folic acid (Penhos and Cardeza, 1952; Penhos, 1955) thus facilitating the action of this hormone and accelerating the formation of nucleic acids and the speed of cellular division. Aminopterin, a folic acid antagonist partially inhibited the action of estradiol benzoate in the *Bufo arenarum* Hensel. This inhibition although partial is significant. Changes in the ovary were not so evident as in the oviduct (Table II).

Testosterone propionate had a clear effect on the increase of weight of testes and the apparition of thumb callosity. These actions were partially inhibited by aminopterin.

The secretions of the oviduct (its role, structure and hormonal control have been widely described by Allende in 1938) are increased when the animal is injected with progesterone (Houssay, 1947). The administration of aminopterin during three weeks produced a statistically significant inhibition (although not total) of the oviduct secretion in progesterone treated toads.

SUMMARY

Testosterone propionate injections of 1 mg, three times a week, produced increase in the weight of testes and apparition of thumb callosity in prepuberal toads.

This effect was partially inhibited by aminopterin (0.1 mg, three times a week).

The stimulating effect of estradiol benzoate (0.5 mg, twice a week) on the oviduct of the prepuberal toad was partially inhibited by aminopterin (0.1 mg, three times a week).

Aminopterin, inhibited the oviduct secretion produced by progesterone in the *Bufo arenarum* Hensel.

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PHYSIOLOGICAL MECHANISMS OF ACCLIMATIZATION

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IN NATURE, as Darwin pointed out, animals become eminently suited to their environment by the process of selection-elimination by death of those unsuited to their environmental conditions. This natural selection, however, is a slow and wasteful process, entailing the death of large number of animals. Lack of adaptation in the improved breeds of livestock results in a slow growth rate, a low reproductive capacity and a high mortality rate.

The skin is a sensory structure placed between the organism and its environment, bringing the former into intimate relationship with the latter. This function is performed by somatoreceptors which are either exteroceptors or proprioceptors. These receptors respond to adequate stimuli of the threshold intensity or above by setting up a single nerve impulse, or more often, a chain of impulses. In the course of adaptation, the external stimuli (environment) are applied at constant intensity for a prolonged period. Thus the impulses generated by the receptors diminish in frequency and may disappear completely.

1. SKIN STRUCTURE AND ADAPTATION

Hairs which are characteristic of mammals exhibit a marked function in the reflection of solar radiation. It is well known that changes in the secretion of endocrine glands mainly the gonads affect profoundly the growth of hair. In dairy cattle, the hair lumen is filled with marrow substance, this is not confined in beef cattle (Csukas, 1949). There are also sex, age and breed differences in respect of the presence and continuation of hair medullation (Duggino & Trotter, 1950). The shedding of hair in cattle and buffaloes is a seasonal phenomenon and may be related to sunlight. The hair of glossy-coated animals are shorter, higher in fat content and of more uniform thickness than the hairs of wooly-coated animals (Bonsma & Pretorius, 1943). The hair of wooly coated cattle tends to grow thick and easily felts when rubbed under pressure in the presence of moisture (Bonsma, 1949 a). Smooth-coated animals have mainly primary hair follicles from which only

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straight hair emerges (Bonsma, 1949 b). Prominent eyebrows and drooping overhanging eyelids serve as a natural protection of the eye against solar radiation.

Animals reared in areas of intense radiation have a larger proportional body surface to body weight than others reared in temperature and cloudy areas. The former develop thick loose hides with vertical skin folds along the neck and sides. The large surface helps the animal to dispose of the surplus heat either by solar radiation or by metabolic processes. The sensitivity of the pilo-muscles are closely associated with the animals ability to move its skin, this moves the hot air surrounling the body and acts as ventilators. The number of blood vessels and capillaries in the skin controls the amount of circulating blood in the skin which is a factor for skin radiation. The proximity of the capillary network to the surface is one of the factors involved.

Skin thickness varies with the breed, sex, age and body region (Yamane and Ono, 1936; Hafez et al., 1955). Body regions exposed to direct solar radiation are thicker than the belly skin. The upper strata of the epidermis are more keratinized in the former regions than the latter. The papillae of the dermis are best developed where the epidermis is thick and hairs are less abundant. The elastic properties of skin as well as the subcutaneous tissues are involved, these have been measured quantitatively (Kirk, 1950).

The pigments responsible for the colour of hair and hide are melanin. The familiar variation in farm animals are for the most part genetically conditioned and result from quantitative and qualitative modifications of the melanin pigments. Seven major genes are involved in this respect (Beadle, 1945). In farm animals the coat colour is due to the pigmentation of hair and that of hide. The colour of pigments of both hair and hide varies with the species as well as the body region. The pigments of hide are yellow and carotene-like in Jerseys (Bonsma & Pretorius, 1943) dark in Zebus (Kelley, 1932), buffaloes (Hafez et al., 1955) and camels (Hafez, 1956). The dorsal and lateral regions are more pigmented than the ventral and leg regions.

In areas of intense radiation such as the tropics, the skull of animals are relatively larger and thicker than in other areas. This is also demonstrated when the skull of negros are compared with these of Europeans. This is a natural protection of the brain and nervous system from the sun stroke due to high light intensities. The body conformation and the proportional size of the body tissues vary with the species. Animals or breeds reared in the tropics have a narrow back and broad frame with a deep body. Other animals (in cloudy areas) have broad backs and a cylindric-shaped body.

2. Physical adaptation

A. *Reflectivity.* — The colour, the type of coat and season of the year influence the reflectivity of sunlight. Reflectivity is higher in Afrikaners than in Jerseys and in summer than in winter (Bonsma & Pretorius, 1943). This is due to darker coat of the Jerseys than in the Afrikaners. Differences in the reflectivity of rays according to season are due to differences in the atmospheric temperature and na-

ture of the light itself. The cream coloured Afrikaner reflects more light than the red Shorthorn.

B. *Absorptivity.* — Through the skin, heat is absorbed in the form of radiant energy or acquired through the mediums of convection and conduction when the environmental temperature are above the skin temperature. At least for moderate light intensities, the kinetic value of the ligth is a logarithmic function of its intensity (Grison, 1943). Also the light absorption of many biological substances is influenced by their temperature (Havemann, 1942). In the tropics, the radiant energy (during 15 hrs in summer) absorbed on the surface of 1000 lb bull amounts to 17000 Kg cal (Riemerschmid, 1943 a), this is as much as three times that produced by the animal itself.

The absorptivity to solar radiation of hides varies with the species, colour (hair & hide) and season. The heat absorptivity of brown coat is much higher than that of white hide while shading reduces absorptivity by about 70 % (Riemerschmid, 1943 b). The solar radiation reaching the animal may be used for the evaporative function of the skin to get rid of the sweat or the drops of rain. Also a part of the solar rays may be radiated from the skin. The two physical factors are influenced by the relative humidity of the atmosphere, angle of incidence of ravs and environmental temperature.

The sebum may function indirectly as a protecting agent against solar radiation. It has a principal reducing agent as acidic (or phenolic) compound (Mackenna et al., 1952). The ether soluble portion of sebum exhibits a bactericidal action as demonstrated by *in vitro* determinations (Miescher et al., 1953). The ultra-violet rays are essential for vitamin D biosynthesis. Deficiency in this vitamin causes incomplete utilization of calcium by the body and leads among other things to the development of rickets in the animals. The role of vitamins in relation to dermatology has been reviewed (Roderer et al., 1950). Meanwhile the superabundance of ultra-violet rays at high levels in the summer in the tropics may have a harmful effect on breeds which have their origin in dull climates.

3. Physiological adaptation

Solar radiation increases the metabolic processes, stimulates certain animal tissues and has a sterilizing influence. These effects are greatly controlled by the nature and quantity of rays imprinting on the animal as well as the species. Body temperature, respiration rate and pulse rate are increased as a result of heat load due to solar radiation. In Egypt (30° N) European breeds are more affected than native cattle (Asker et al., 1952). This may be due to the different geographical origin of the animals. Animals reared in an environment different to that of their origin are less tolerant to heat load (due to solar radiation) than others reared in a locality similar to their origin.

The buffalo has a better cooling mechanism under shade than the cow as shown in India (Mullick & Kehar, 1951). Sprinkling for 3 minutes followed by shading for two hours indoors is more effective in reducing respiratory and circulatory functions especially in European breeds. It seems that the nature of solar radiation is also involved here.

The effects of heat load on the physiology and psychology of farm animals have been extensively discussed and augmented (Phillips, 1948; Lee & Phillips, 1948; Brody, 1948; Findlay, 1948).

4. Practical application

It can therefore be seen how important it is that animal breeders give adequate attention to the selection of appropriate coat and hide colour. A white, yellow or red coat with a dark hide is the ideal combination to render the animal resistent to intense radiation. This is well illustrated in the Indian cattle breeds, the Afrikaner cattle (Bonsma, 1949 a, b), the Arabian horses (Oliver, 1938; Ware, 1941), buffaloes (Hafez et al., 1955) and camels.

In cattle, black breeds are best adapted in those regions where short-wave radiation is intense, for example, at high altitudes and where mist frequently occurs. Shade or cloudiness is an essential condition for dark colours to operate efficiently. In pigs, which have little hair, the penetration of rays into the tissues underlying the skin causes the greatest trouble. Pigs with coloured skin are therefore preferred in strong sunlight areas where there is but little shade. Some individual pigs of the white breeds seem to be more resistant than others, but the exact cause for this has not been determined.

The characters associated with increased adaptability to intense radiation are for the greater part dominant. Several attempts have been carried out to involve new breeds for the tropics combining high adaptability and productivity. In cattle, the Santa Gertrudis has been developed in U. S. A. (Rhoad, 1949) and another breed out of Shorthorn X Afrikaner crosses is now to be established in S. Africa (Joubert, 1953). Since most of the bacon breeds of pig in the world are white, there has been a need to produce a coloured bacon breed for sunny climates. The Minnesota N° 1 developed by Winters is a cross between the white Danish Landrace and the red Tamworth. In New Zealand, the Lincoln Red breed is a cross between the Tamworth and Large White (Hammond, 1949). The existing black breeds have been also improved such as the Large Black of South Africa and the Canadian Berkshire of Australia.

Solar radiation as controlled by daylength affects the periodicity and intensity of grazing. The daylength varies with the latitude and season. Thus the "grazing day" which may be approximately termed "dawn to dusk" is reduced in high latitudes and during the winter months. This has an interesting effect on compressing the grazing periods together and reducing the time spent idling during the day. Grazing patterns in relation to season have been observed under experimental conditions (Tayler, 1951).

Well adapted breeds are capable of grazing throughout the day even under very intense radiation whereas less adapted breeds seek the protection of trees and bushes for the relaxing of mind and muscle. The feeling of safety that such a partial protection from light induces is not usually appreciated as a biological factor and accordingly hardly ever taken into consideration in our husbandry methods.

Summary

Different mechanisms of adaptation are recognized in farm animals, mainly the skin anatomy, the physical characteristics of hides and the physiological processes of the body. Hair shedding is affected by daylight. The prominence of eyebrows and their bony ridges provide natural protection from sunlight. Intense irradiation is associated with large skin surface and sensitivity of pili-muscles. Skin conductivity depends on its thickness, keratinization, pigmentation, nature of skin strata, elastic properties and subcutaneous fat. Body conformation and size thickness of skull vary with the degree of irradiation. Reflectivity and absorptivity of skin vary with the species pigmentation and the season of the year.

The metabolic rate is increased by radiation and decreased by shading. Differences in response amongst species are related to the geographical origin and type and duration of radiation or shading. Beyond the threshold level of exposure, pathological conditions occur.

A light-colour coat with a dark hide is ideal for the tropics and subtropics. Breeds of pigs with coloured skin are required for the tropics. Grazing patterns in relation to adaptation are considered.

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EXCRETION RATES OF OESTROGENS IN FARM ANIMALS

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A KNOWLEDGE of the role and level of ovarian hormones in the animal body is of no little importance in solving certain problems in the reproductive physiology. Oestrogens have been detected and isolated in several body organs and fluids such as the ovaries and liquor folliculi of sheep (Allen and Doisy, 1923), human corpus luteum (Allen et al., 1925), wall of corpus luteum (Zondek and Ascheim, 1925, 1926), maternal placenta (Doisy et al., 1924), foetal placenta, amniotic fluid (Parkes, 1928), blood of oestrus and pregnant females (Frank et al., 1925), testes and adrenal cortices (Doisy et al., 1924). Oestriol has not been isolated from any endocrine organ other than the placenta (Curtis et al., 1934). The distribution of oestrogens is not only confined to vertebrates but also occur in marine invertebrates (Donahue, 1940).

It was hoped in this paper to review and augment literature on the biosynthesis, metabolism, and assay methods of the natural oestrogens with special reference to its excretion in farm animals.

Biosynthesis & Metabolism. — Natural occuring oestrogens are phenolic steroids which are characterized by the basic cyclopentanoperhydrophenanthrene nucleus. They are oestradiol, oestrone, equilin, equilenin, their alpha and beta-dihydroderivatives, and oestriol (hydrated oestrone). It is believed that the cholesterol esters and fatty acids together with ascorbic acid are considered as precursors of oestrogens (Classon et al., 1948). The accepted scheme of conversion of oestradiol as the principal ovarian oestrogen is

$$\underset{\text{oestradiol}}{\overset{(A)}{\rightleftarrows}} \underset{\text{oestrone}}{\overset{(B)}{\rightarrow}} \text{oestriol}$$

That 16-ketosterone may be an intermediate in the process (B) has been suggested (Stimmel et al., 1948). The skeleton, the blood (Werthessen et al., 1950) as well as various other body tissues (Perlog and Fuhrer, 1945) are involved in oestrogen metabolism. Pearlman and Rakoff (1949)

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have shown that oestrogen metabolites are excreted into human pregnancy bile and hence into the gut via the enterohepatic circulation, chiefly as conjugates. These findings have revealed the role of the liver in oestrogen metabolism; mainly conjugation (Crepy, 1947) and conversion (Pearlman and De-Meio, 1949). Various *in vitro* studies with liver mince and liver fractions suggest that the hepatic oestrogen-inactivating system involves cozymase, flavin-adenine-nucleotide and cytochromec (DeMeio et al., 1948 and Furlong et al., 1949).

Assay Methods. — Methods of estimation of oestrogenic potency can be classified into physicochemical and biological. The physicochemical assay depends upon the presence of the phenolic group at position 3, the presence of a ketonic group or an alcoholic group at position 17. The physicochemical assay has been based on gravimetric (Hughes, 1941), polarographic (Wolf et al., 1941), photometric (Friedgood and Garst, 1948), infra-red (Furchgott et al., 1946; Carol et al., 1948) and fluorimetric methods (Finkelstein et al., 1947). The Kober (1931), the Zimmerman (1935) and the fluorimetric methods are considered more satisfactory for testing partially purified non-crystalline extracts from the phenolic fractions of the urine. These previous methods can be used directly with conjugated oestrogen fractions.

The bioassay methods are based on the vaginal smear technique using spayed mice or rats (Allen and Doisy, 1923). Many modifications have been introduced such as the frequency of injections, the number of days injected, the route of administration, the solvent used and the experimental test animal. However, there is a very serious doubt as to the reliability of the chemical assay methods, except possibily as applied to human urine of late pregnancy in which the excreted oestrogens may be as much as 10 to 20 mg per day; and even in this case, overestimation by the chemical assay is quite common (Emmens, 1950).

Species Differences In Excretion Rate. — Early in 1927 Ascheim and Zondek recognized the presence of oestrogens in the urine of cows. Oestradiol and oestrone are the forms of oestrogens detected in the cows' urine (Hansel, 1954). It is well established that oestrogen level in cows' urine is comparatively low than that of the mare or woman. In the cow, the daily excretion of oestrogens in the urine during the first hundred days of gestation is some 50 r. u. which is followed by a steady increase until parturition (Nibler and Turner, 1929). In late pregnancy, the cow excretes some 3000 to 8000 r. u. of oestrone per kilogram of solids in the faeces (Levin, 1945).

The urine of pregnant buffaloes contains oestradiol and oestrone but no oestriol (Hafez and Attar, 1955). The first detectable amount of oestrogens in the buffaloes' urine is during the third month of gestation at an average level of 37.5 μg per liter, this is followed by an increase until the eight month (Hafez and Attar, 1955). During the ninth month, the hormone level is maintained at an average of 193 μg per liter of urine, and in the last fifteen days prior to parturition no oestrogens are to be detectable.

In the ewe, the daily level of oestrogens is consistently low until late pregnancy, it ranges from 0.2 to 3 μg oestradiol and 1.5 to 20 μg

TABLE I

The level of oestrogens in farm animals

Species	Source	Quantity	Condition	Investigator
Cow	Urine	11 ru per liter	Non-pregnant	Turner et al. (1930).
	Urine extracted without hydrolysis	5000-6000 ru per liter	End of pregnancy	Nibler and Turner (1929).
	Hydrolyzed urine	17.000 mu per liter	End of pregnancy	Barrie et al. (1935).
	Faeces	5000-8000 ru per kilogram of solids	Late pregnancy	Levin (1945).
Buffalo	Urine	340.60 ug per liter	Eigth month of pregnancy, Peak	Hafez and Attar (1955).
Mare	Urine	5000 mu per liter	Non-pregnant	Glud et al. (1933).
	Urine	50-200 mg per liter	Peak of pregnancy	Lozinski (1953).
Stallion	Urine	1.5 mg per liter		Deulofeu and Ferrari (1934).
	Urine	35.000-300.000 mu per liter		Haussler (1934).
Ewe	Urine	0.2-3μg oestradiol, 1.5-20 μg oestrone per day	Last few weeks of pregnancy	Beck (1950).
	Faeces	1-20μg oestradiol, 1-100μg oestrone per kilogram of solids		"

oestrone, while the daily levels of faecal oestrogens at the same stage of gestation is 1 to 20 μg oestrone, 1 to 100 μg estradiol, and no detectable amounts of oestriol (Beck, 1950).

In the non-pregnant mare there are two peaks of urinary oestrogen excretion, the first during oestrous and the second between the tenth to fifteenth day of the cycle (Mayer et al., 1940). The urine of pregnant mare, however, is a rich source of oestrogen (Zondek, 1934); this finding is confirmed by Beall and Edson (1936), Cole and Saunders

(1935), Edson and Heard (1939), Glud et al. (1933), Hart and Cole (1934), Kober (1935) and Lozinski et al. (1942). In the mare, the oestrogen level increase appreciably until the third or fourth month of gestation, hence the oestrogen content of urine is of no diagnostic value for pregnancy until some two months after the jump of gonadotrophins

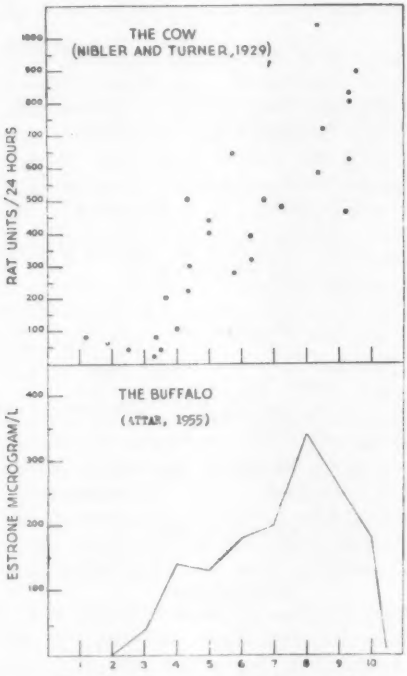

FIG. 1. — *Urinary estrogens excreted during cow and buffalo pregnancy.*

in the blood. The oestrogens reach a peak of 50 to 200 mg per liter of urine about the seventh month of pregnancy, and gradually falls off at term (Bates, 1953 and Lozinski, 1953). The high oestrogen content of the stallion is not paradoxical as it may at first appear, for the steroids excreted in the urine are products of metabolism and functioning hormones.

The urinary oestrogens in the sow is present for a short period in early pregnancy, approximately the 20th day (Roth et al., 1941).

In the woman the total oestrogen of the urine increases gradually after the first week of pregnancy, reaches a peak shortly before parturition, and disappears abruptly a few days *post-partum*. The total amount of conjugated oestrogens begins to fall shortly before parturition, whereas the amount of non-conjugated physiologically active oest-

rogen undergoes a relative increase at this time (Cohen and Marrian, 1935).

The urinary oestrogens in different species of farm animals is tabulated (Table) and illustrated graphically (Figure 1 and 2). The forms and the level of the oestrogenic hormones in the excreta vary

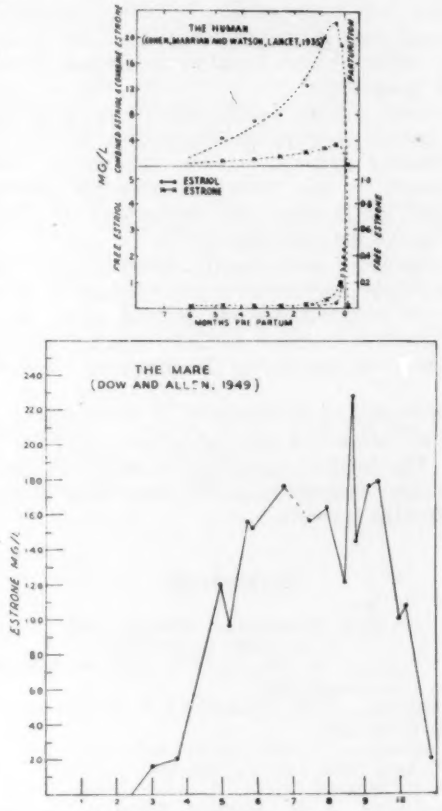

FIG. 2. — *Urinary estrogens excreted during human and mare pregnancy.*

among the different species of farm animals, as well as the route through which these hormones are expelled from the body. The cow and the ewe excreted oestrogens mainly in the faeces, while in the mare these hormones are excreted mainly via the urinary tract. These species differences are still obscure and may be related to certain evolutionary trends in the endocrine system as well as the metabolic activity of the hormones. Further investigations are needed in the field of the steroid chemistry and metabolism.

There are considerable difficulties in the present physicochemical assay methods since the level of excreted oestrogens is comparatively low even after the administration of massive doses of exogenous hormones.

SUMMARY

1) Natural occuring oestrogens, oestradiol, oestrone, equilin, equilenin, their alpha and beta-dihydroderivatives, and oestriol are phenolic steroids which are detected and isolated in several body tissues during non-pregnancy and pregnancy.

2) The cholesterol esters, fatty acids, and ascorbic acid are considered as precursors of oestrogens. Oestrogen metabolism, mainly conjugation (esterification) and conversion (oestradiol, oestrone, oestriol) are carried out mainly in the liver, the skeleton and blood being also involved. Oestrogen metabolites are excreted via the faeces in the cow and ewe and via the urine in the mare.

3) The estimation of oestrogenic potency is based on physicochemical assay involving gravimetric, polarographic, photometric, infrared and fluorimetric methods which depend upon the presence of a phenolic group at position 3 and a ketonic or alcoholic group at position 17. Bioassay methods are based on the vaginal smear technique using spayed rodents.

4) The major forms of oestrogens in the excrets of the cow, the ewe, and in buffaloes urine are oestradiol and oestrone while no oestriol is to be detectable. The level of excreted hormones varies with the species and stage of pregnancy (tabulated), this may be related to evolutionary trends in the endocrine system.

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INFLUENCE OF DL-GLYCERALDEHYDE AND OF L-SORBOSE-1-PHOSPHATE ON GLYCOGEN SYNTHESIS FROM GLUCOSE BY RAT LIVER SLICES *

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INTRODUCTION

MENDEL (1929) DISCOVERED that DL-glyceraldehyde in concentration of 1 mM/L inhibited the anaerobic formation of lactic acid from glucose by Jensen Sarcoma and rat brain slices. Subsequently, many other workers have demonstrated such effect in several other tissues (Holmes, 1934; Adler and col., 1937; Rudney, 1949). The inhibitory effect of DL-glyceraldehyde can be ascribed entirely to the L-component (Needham and Lehmann, 1937).

As glyceraldehyde has no effect on glycolysis of polysaccharides or phosphorylated sugars it has been postulated that hexokinase reaction is the site of action of glyceraldehyde (Adler and col., 1937; Stickland, 1941).

Evidence has been obtained that the L-glyceraldehyde condenses with dihydroxyacetone phosphate through the aldolase reaction to yield L-sorbose-1-phosphate (S-1-P). This has been demonstrated to be the direct inhibitor of hexokinase reaction (Lardy and col., 1950).

In previous papers, it has been demonstrated that glucose promotes glycogen synthesis much more than glucosephosphates do (Niemeyer and col., 1953; Cruz-Coke and col., 1954; Niemeyer and Figueroa, 1956). It was suggested that glycogen is formed from glucose in rat liver by a pathway that excludes hexokinase reaction. This report informs on the effect of L-sorbose-1-phosphate and DL-glyceraldehyde ** on the reaction glucose → glycogen. If glycogen synthesis is inhibited it may indicate that hexokinase reaction is required. On the contrary, if glycogen synthesis is not affected, hexokinase reaction presumably is not important in glycogen formation.

EXPERIMENTAL

Male rats weighing from 200 to 250 g were used. The animals were

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** We are indebted to Dr. H. A. Lardy for making available samples of DL-glyceraldehyde and L-sorbose-1-phosphate.

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sacrificed by a sharp blow on the head and the liver or the diaphragm were excised and put inmediately in cold Krebs phosphate solution (Krebs, 1933). The liver slices were prepared free-hand with a razor blade and kept in oxygenated cold Krebs solution modified to contain less calcium. Diaphragm was cut in little pieces and put in the same conditions as liver. About 100 mg of liver slices or 50 mg of diaphragm were placed in Warburg vessels containing 2 ml of Krebs solution with the additions indicated in the respective tables. After oxygenation and thermal equilibrium, one vessel from each experimental condition was removed and tissue "initial glycogen" was measured. After 60 minutes of incubation the tissue "final glycogen" was measured in the other vessels. Initial glycogen was not determined in the diaphragm. Glycogen was determined by the procedure of Walaas and Walaas (1950) with Somogyi (1945) and Nelson (1944) reagents.

RESULTS

Glycogen breakdown is always observed in liver slices under the experimental conditions used. When glucose is added as a substrate, the disappearance of glycogen from the tissue decreases. The rate of glycogen breakdown may be considered a dynamic equilibrium between synthesis and degradation. Since it has been demonstrated that 2.4-dinitrophenol inhibits the effect of glucose on glycogen disappearance (Niemeyer and col., 1956), it may be postulated that this effect is the consequence of a higher synthesis of glycogen. Therefore, the modifications induced by DL-glyceraldehyde and L-sorbose-1-phosphate may be considered as an influence on the synthesis of glycogen from glucose.

DL-glyceraldehyde effect. — Table I shows that DL-glyceraldehyde

TABLE I

Influence of DL-glyceraldehyde on glycogen content of rat liver slices incubated in Krebs saline, with and without glucose

Exp. Nº	Initial Glycogen %	Residual Glycogen (%) *			
		No substrate	Glyceraldehyde	Glucose	Glyceraldehyde + Glucose
1	1.53	5	9	18	21
2	2.80	8	7	14	13
3	4.10	34	40	51	52
4	4.34	25	27	58	67
5	4.59	43	50	61	71

Glucose concentration was 30 mM/L; Glyceraldehyde was 10 mM/L, except in experiment 2 which was 20 mM/L.

* Per cent of initial glycogen remaining at the end of 60 minutos of incubation.

does not exert any appreciable modification on glycogen breakdown in rat liver slices, suspended in a medium with glucose or without added substrates. On the contrary, DL-glyceraldehyde inhibits glycogen synthesis from glucose in diaphragm (Table II), considered to be represen-

TABLE II

Influence of DL-Glyceraldehyde on Glycogen content of rat diaphragm incubated in Krebs saline, with and without glucose

Exp. Nº	Final Glycogen (%)			
	No substrate	Glyceraldehyde	Glucose	Glyceraldehyde + Glucose
1	0.04	0.04	0.16	0.05
2	0.14	0.16	0.33	0.20
3	0.17	0.19	0.38	0.25
4	0.20	—	0.29	0.21
5	0.20	0.27	0.35	0.28
6	0.21	0.16	0.44	0.30

Glucose concentration was 30 mM/L; Glyceraldehyde was 10 mM/L.

tative of muscular tissue. The arithmetic mean of the difference between the final glycogen in the presence of glucose, with and without DL-glyceraldehyde was 0.11 ± 0.01 % ($P < 0.001$).

L-sorbose-1-phosphate effect. — S-1-P added to the incubation medium does not modify glycogen formation neither in rat liver slices nor in diaphragm (Tables III and IV).

TABLE III

Influence of L-sorbose-1-phosphate on glycogen content of rat liver slices incubated in Krebs saline, with and without glucose

Exp. Nº	Initial Glycogen %	Residual Glycogen (%)*			
		No substrate	S-1-P	Glucose	S-1-P + Glucose
1	1.04	10	8	15	15
2	2.92	29	35	50	51
3	3.64	32	28	45	44

Glucose concentration was 30 mM/L; S-1-P was 1 mM/L in experiments 1 and 2; and 2 mM/L in experiment 3.

* Per cent of initial glycogen remaining ot the end of 60 minutes of incubation.

TABLE IV

Influence of L-sorbose-1-phosphate on glycogen content of rat diaphragm incubated in Krebs saline, with and without glucose

Exp. Nº	Final Glycogen (%)			
	No substrate %	S-1-P %	Glucose %	S-1-P Glucose %
1	0.12	0.06	0.22	0.31
2	0.08	0.10	0.18	0.21
3	0.31	0.45	0.55	0.66

Glucose concentration was 30 mM/L; S-1-P was 1 mM/L in experiments 1and 2; and 2 mM/L in experiment 3.

Discussion

That glycogen synthesis from glucose would not necessarily need the hexokinase reaction in rat liver may be inferred from the present results, although they are not conclusive. In fact, DL-glyceraldehyde inhibits glycogen synthesis in diaphragm, but it does not interfere in the synthesis of glycogen by liver slices. The lack of action on liver could be the consequence of aerobic conditions, which may counteract the effect of glyceraldehyde by permitting the oxidation of triosephosphate and preventing thus the condensation that leads to S-1-P. However this does not explain the difference between liver and diaphragm.

To obviate this difficulty in the interpretation of the action of glyceraldehyde, S-1-P itself was used to inhibit hexokinase reaction. With this substance, no effect was observed, even at a concentration ten to twenty times higher than that used by Lardy and col. (1950). The increase of oxygen consumption (10 ± 2.4 %), brought about by S-1-P on liver slices is interpreted as an indication that the substance has entered into the cell. It is rather difficult to explain why S-1-P does not exert any significant effect upon diaphragm, if the results obtained with glyceraldehyde are considered; perhaps the low permeability of muscle cell membranes to hexosephosphates, might be involved.

The results presented in this paper, although not conclusive, would favour the hypothesis that hexokinase reaction does not have an important role in glycogen formation from glucose by rat liver slices.

Summary

DL-glyceraldehyde and L-sorbose-1-phosphate do not affect glycogen synthesis from glucose by rat liver slices. In contradistinction, DL-glyceraldehyde inhibits glycogen formation by rat diaphragm. No effect was observed with S-1-P in the last tissue.

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INFLUENCE OF GLYCOGEN CONTENT ON THE EFFECT OF 2,4-DINITROPHENOL ON GLYCOGEN METABOLISM IN RAT LIVER SLICES *

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INTRODUCTION

IN PREVIOUS PAPERS data were presented which demonstrated that the effect of DNP ** on the oxygen uptake by rat liver slices varied with the glycogen content of the tissue (Niemeyer and Figueroa, 1954, 1955). According to the interpretation given to those experiments it could be expected that liver glycogen might influence the effect of DNP upon some of the processes of biosynthesis which are presumably affected by the drug.

In this report evidence is presented which shows that glycogen content of rat liver slices actually modifies the effect of DNP on glycogen synthesis from glucose or fructose.

EXPERIMENTAL

Male rats weighing from 200 to 250 g were sacrificed after fasting periods varying from 0 to 20 hours. Livers were excised and slices were prepared with a razor blade and kept in oxygenated cold buffer solution. About 100 mg of slices were put in conventional Warburg vessels to which 2 ml of saline buffer had been added. The flasks were oxygenated and after ten minutes of thermal equilibrium, one of the three vessels of each experimental condition was removed and tissue "initial glycogen" was determined. After 60 minutes of incubation at 37.7° C, the tissue "final glycogen" was measured in the two remaining flasks. The procedure of Walaas and Walaas (1950) with Somogyi (1945) and Nelson (1944) reagents was used. Two buffers were employed: the Krebs-phosphate medium (Krebs, 1933) modified to contain less calcium, and a solution with high concentration of intracellular cations (KH solution) with the following composition: 0.154M KCl 70 ml; 0.11M $MgCl_2$ 18 ml; 0.11M $CaCl_2$ 4 ml; 0.10M K_2HPO_4 adjusted to pH 7.5, 8 ml.

Glucose and fructose were used as substrates at 30 mM/L concen-

* Aided by a grant from the Williams-Waterman Fund of the Research Corporation, New York. The data of this paper were presented at the Sociedad de Biología, Santiago, December 14, 1954.

** DNP will designate 2.4-dinitrophenol and ATP, adenosinetriphosphate.

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tration. DNP (Amend) twice recrystallized, was dissolved in isotonic NaCl, or KCl, and neutralized. Part of the NaCl or KCl in the Krebs solution or the KH solution respectively were replaced by the DNP solutions so as to obtain the desired concentration (0.1 mM/L).

RESULTS

Effect of DNP on glycogen breakdown in liver suspended in media without substrates. — When rat liver slices are suspended in media without substrates, glycogen breakdown is always observed. The rate of breakdown is lower in KH solution than in Krebs medium, as it is shown on Table I.

TABLE I

Glycogen evolution in rat liver slices incubated in two different media, without substrate or with glucose (30 mM/L)

Medium	Substrate	Nº of Exp.	Residual glycogen (%) *
Krebs	None	54	23 ± 2.2
	Glucose	54	40 ± 3.2
KH	None	22	57 ± 4.6
	Glucose	16	92 ± 10.6

*Per cent of initial glycogen remaining ah the end of 60 minutes of incubation. Arihmethic mean and standard error.

Glycogen breakdown was not significantly affected by DNP in Krebs medium when the initial glycogen content was low. In the presence of initial glycogen levels ranging from 2 to 5 %, DNP enhanced glycogen breakdown, so that final glycogen became equivalent to 80 % of that of the control slices incubated without the drug (Fig. 1).

The effect of the pharmaco was more pronounced in KH medium. In fact, final glycogen with DNP was about 10 % of that of the controls without DNP, when the initial glycogen was about 1 %. The DNP effect diminished when initial glycogen was lower or higher than this figure (Fig. 1).

As the rate of glycogen synthesis may be considered a dynamic equilibrium between synthesis and degradation, it may be supposed that the difference in the final glycogen content observed in slices suspended in Krebs and KH solutions, is due to the occurrence of a higher rate of resynthesis of glycogen from liberated glucose in the KH medium. Since the priming effect of ATP is required solely for the synthesis, the proposed interpretation may well explain why the effect of DNP is so much greater in slices incubated in KH solution.

Influence of DNP on the effect of hexoses on glycogen breakdown. — Addition of glucose or fructose inhibited glycogen breakdown in both media (Table I), but a net synthesis of glycogen was occasionally

observed only with KH saline. The effect of the hexoses was not apparent at the beginning of the incubation period, when "initial glycogen" was measured.

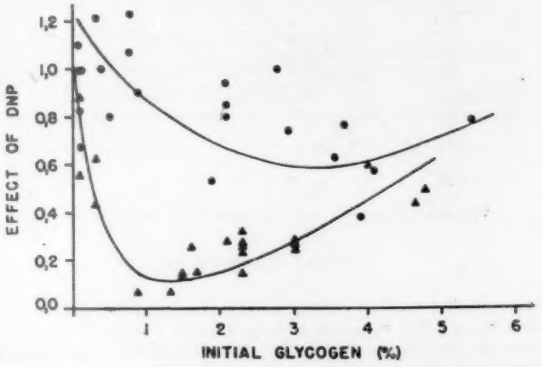

FIG. 1. — *Influence of the initial glycogen content on the effect of DNP upon the glycogen disappearance from rat liver slices incubated in media without substrates. The effect of DNP is expressed as the quotient between residual glycogen after 60 minutes of incubation with and without the pharmaco. The circles correspond to experiments perfomed in Krebs medium and the triangles in KH medium.*

DNP depressed the action of hexoses on glycogen breakdown, the extent of its effect depending on the glycogen content of the tissue. The existence of two variables, the presence or absence of hexose and the presence or absence of DNP, complicates the analysis of the results. In order to perform this analysis, the final glycogen content of liver slices in each experimental condition will be denominated as follows:

a = in saline without addition
b = in saline with hexose
c = in saline with DNP
d = in saline with DNP and hexose

The relation $Z = \frac{d - c}{b - a}$ gives the influence of DNP on the effect of the hexose added to the medium. If the value of $Z = 1$, it means that DNP does not interfere on the hexose effect. If DNP depresses hexose action, $Z < 1$; whilst if DNP enhances it, $Z > 1$.

It may be seen in Figs. 2 and 3 that the value of Z depends on the initial liver glycogen in both incubation media. Thus, Z is small when initial glycogen is low, and it approaches the unity as glycogen increases. In other words, DNP inhibits the hexose effect on glycogen breakdown markedly when glycogen content is low and slightly when glycogen content is high. The statistical analysis reveals that there is a significant correlation between the values of Z and the glycogen content. For glucose, $r = 0.76$ ($P < 0.01$) in Krebs medium and $r = 0.73$ ($P < 0.05$)

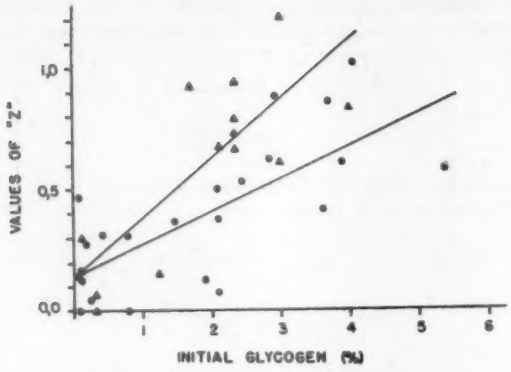

FIG. 2. — *Influence of the initial glycogen content on the effect of DNP upon the action of hexoses on the residual glycogen from rat liver slices after 60 minutes of incubation in Krebs medium. Circles represent experiments with glucose as a substrate and triangles with fructose. For the meaning of "Z", see the text.*

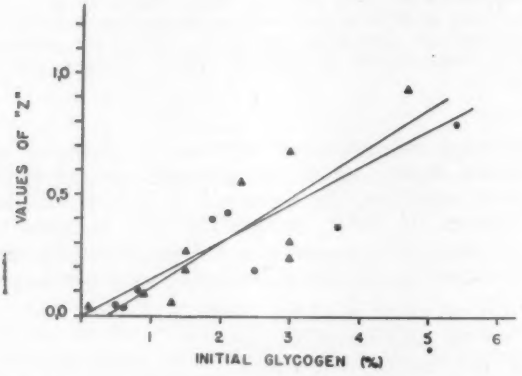

FIG. 3. — *The same as Fig. 2, but incubation of the liver slices was performed in KH medium.*

in KH solution; and for fructose, $r = 0.82$ ($P < 0.01$) in Krebs saline and $r = 0.78$ ($P < 0.01$) in KH medium.

Influence of DNP on the release of glucose by liver slices. — The data presented in Table II show that DNP does not modify significantly the release of glucose by liver slices incubated in Krebs solution without substrate. This lack of effect of DNP occurs even in slices with low glycogen content. It is important to stress the fact that the amount of glucose released by the slices with a low glycogen content cannot be accounted for by the initial glycogen of the tissue.

This must be due to the formation of glucose from non-glucidic precursors, a process which, in the light of our results, appears not to be affected by DNP.

TABLE II

Influence of 2.4-dinitrophenol (0.1 mM/L) on the release of glucose from rat liver slices incubated in Krebs solution

Exp. Nº	Initial Glycogen %	(mg/100 mg tissue/hour) Glucose released * Without DNP	With DNF
1	0.29	1.07	1.17
2	0.38	0.91	0.75
3	0.61	0.91	0.81
4	0.94	1.10	1.12
5	2.12	1.83	1.74
6	2.52	1.39	1.04

(*) The statistical analysis shows trat there is no difference between glucose released to the medium in the presence and in the absence of DNP ($P > 0.01$).

Discussion

Niemeyer and Figueroa (1954, 1955) have pointed out in previous papers that the effect of DNP on the oxygen uptake by rat liver slices depends on the glycogen content of the tissue. Thus, if the glycogen content is low, the drug depresses oxygen consumption, whilst, if it is high, DNP stimulates tissue respiration. When substrates which do not require the priming action of ATP in order to be oxidized are added to the incubation medium, DNP no longer inhibits oxygen uptake by rat liver slices with low glycogen content, but it enhances it in most cases. In the presence of substrates that do require the priming action of ATP, DNP continues to show its inhibitory effect upon respiration. On the other hand, if the glycogen content of the slices is high, DNP always stimulates oxygen uptake, no matter what the substrate may be.

It was postulated that the high glycogen content could account for a greater production of ATP in reactions non-sensitive to DNP, thus maintaining a level of ATP which would be adequate for the activation of those substrates which require it. In fact, the aerobic production of lactic acid by rat liver slices is directly proportional to the initial glycogen (Niemeyer et al., 1956). This means that there is a greater breakdown of glycogen when the initial glycogen is high and consequently, that there is an enhanced formation of phosphoglyceryl-phosphate and phosphoenolpyruvate which represent the creation of high energy phosphate bonds in processes which are not interfered by DNP (Lipmann and Kaplan, 1947). Further support is afforded by the observation that if ATP is added to the medium in which slices with low glycogen content are incubated, the inhibitory effect of DNP on respiration is counteracted (Niemeyer and Figueroa, 1955).

The hypothesis that glycogen represents a source of ATP non sensitive to DNP may also explain the results presented in this paper, which show that a high level of initial glycogen antagonizes the inhibitory effect of DNP upon the synthesis of glycogen from glucose and fructose.

It seems interesting to point out that glycogen has revealed a similar action against the inhibition produced by DNP on the synthesis of p-aminohippurate by rat liver slices (Niemeyer, González, Figueroa and Coghlan, unpublished data).

It may be supposed that glycogen exerts a similar protective role in tissues submitted to anaerobiosis, a condition in which the production of ATP in the process of oxidative phosphorylation is blocked by the absence of oxygen. Thus, the above mentioned hypothesis would offer an explanation for the fact observed by Craig (1943) that the metabolic changes induced by anaerobiosis on rat liver slices are more pronounced in tissue obtained from fasted rats than in slices excised from liver of fed rats. It may also account for the observation that fructose will enhance the production of lactic acid in anaerobiosis only in rat liver slices obtained from fed rats (Rosenthal, 1929; Dickens and Greville, 1932).

The inhibitory effect of DNP is greatest in KH medium at an initial glycogen content of 1 % and decreases progressively as the glycogen level rises. This result is in perfect agreement with the interpretation offered in previous paragraphs. Strangely enough, the effect of DNP also diminishes if initial glycogen falls below 1 %. In Krebs' medium, as well, the action of DNP is insignificant when liver glycogen is very low. We have found a rather possible explanation for these facts. The data presented in Table II showed that slices with low initial glycogen produce glucose at the expense of non glucidic precursors and that this process is not interfered by DNP. It seems possible that glycogen, as well as glucose, may be formed from such precursors in reactions unaffected by DNP, and that this glycogen may be responsible of the anomalous fact observed in slices with very low glycogen content.

Summary

DNP (0.1 mM/L) enhances glycogen breakdown in rat liver slices incubated in saline without substrates. Glucose and fructose (30 mM/L) inhibit glycogen breakdown, and this action is partially counteracted by DNP. The intensity of the effect of the drug is inversely proportional to the glycogen content of the liver slices.

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PARTICIPACION DE LA ANEMIA EN LA MUERTE POR BARTONELOSIS AGUDA

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EN UN TRABAJO anterior (1) se estudió el síndrome hemolítico provocado por la esplenectomía en la rata blanca portadora de bartonellas, causante de un elevado porcentaje de mortalidad, y se consideró la posibilidad de que ésta obedeciera a la intensa y brusca anemia consecutiva a la hemólisis. Experimentos completados con posterioridad arrojaron dudas sobre esa posibilidad al permitir comprobar que animales anemizados por sangría podían tolerar cifras muy bajas de glóbulos rojos sin sufrir mayores trastornos y conservando una gran capacidad de recuperación.

En este trabajo se estudia la participación de la anemia en la muerte por bartonelosis aguda.

MATERIAL Y MÉTODOS

Se usaron 109 ratas blancas adultas, de ambos sexos, distribuidas en cuatro grupos: *Grupo I*: esplenectomizadas sin ningún tratamiento. *Grupo II*: esplenectomizadas y anemizadas. Luego de la esplenectomía se las sangró dos o más veces diarias por la cola, previo calentamiento, hasta lograr que sus cifras normales (7 a 8 millones) descendieran alrededor de 2 a 4 millones el tercer día de la operación. En ese momento se suspendió la sangría. *Grupo III*: esplenectomizadas, anemizadas y transfundidas. Fueron tratadas igual que el grupo anterior pero el día tercero, cuando se encontraban con cifras de hematíes de 2 a 4 millones, recibieron una inyección endovenosa de 4 cc de glóbulos rojos homólogos centrifugados y desplasmatizados, con lo que las cifras ascendieron de inmediato a más de 6 millones. *Grupo IV*: sangradas y transfundidas, sin esplenectomía (testigos de compatibilidad). Recibieron el mismo tratamiento que los del grupo III pero no fueron esplenectomizadas.

Catorce animales del primer grupo y todos los de los otros tres tuvieron control hematológico diario o cada dos días hasta la muerte o hasta el día 10º de la operación los que sobrevivieron.

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Resultados

Grupo I. — Todos los animales de este grupo (65) mostraron los síntomas típicos del síndrome hemolítico, y se observó entre ellos una elevada mortalidad que alcanzó al 79.5 % el día 10º. Se eligió este día para computar el porcentaje de muertes porque anteriormente había podido comprobarse que la gran mayoría de los animales que mueren, lo hacen antes de los diez días.

Grupo II. — En el grupo de los esplenectomizados y anemizados por sangría la inundación bacteriana tuvo lugar en un momento en

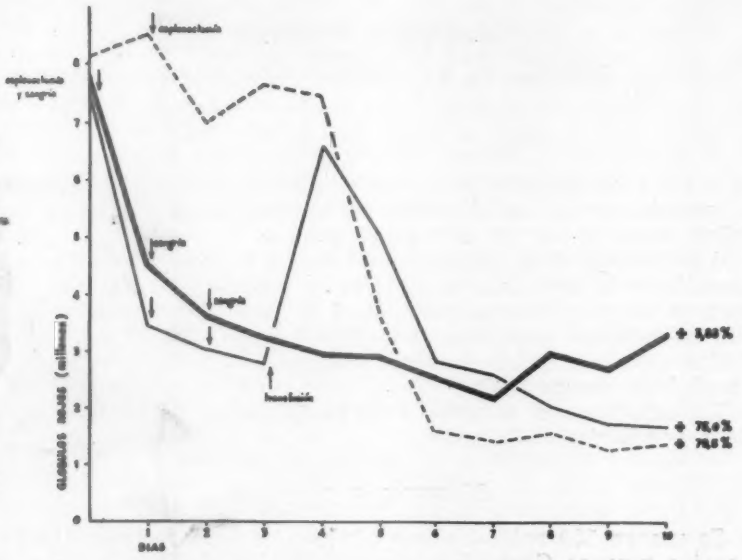

Fig. 1. — *Curva de glóbulos rojos y porcentaje de mortalidad inmediata en: esplenectomizados testigos (línea de puntos), esplenectomizados y sangrados (línea gruesa) y esplenectomizados, sangrados y transfundidos (línea fina). Desde los días 3 a 8, bartonellas visibles en los frotis.*

que por la repetida sangría los niveles de glóbulos rojos estaban muy disminuídos.

Los animales de este grupo (26) no mostraron signos del síndrome hemolítico excepto la palidez ya manifiesta desde las primeras sangrías, y atribuible a la anemia por sí misma, y conservaron un excelente estado general. Las cifras de glóbulos rojos se mantuvieron bajas después de suspendida la sangría, o descendieron todavía más, comenzando a recuperarse recién unos seis días más tarde, en momentos en que testigos sin infección, sangrados en la misma forma, se encontraban ya alrededor de los cinco millones.

En los frotis de todos los animales de este grupo se constató la presencia de abundantes bartonellas desde el día 3º al 7º u 8º. No

obstante la indudable bacteriemia, ninguno de los animales murió en el término habitual de 4 a 7 días, y sólo más tarde, cuando las cifras de glóbulos rojos se aproximaban a lo normal algunos de entre ellos experimentaron un nuevo empuje del proceso, volviendo a mostrar el cuadro infeccioso, esta vez con todo el cortejo sintomático del síndrome hemolítico y muerte.

La mortalidad el día 10º fué de 3.85 %.

χ^2 de mortalidad el día 10º

	Total	*Viven*	*Mueren*
Esplecnectomizados sin sangría	65	14	51
Esplecnectomizados sangrados	26	25	1

$\chi^2 = 39.05$

$\chi^2_{(0.001)} = 10.827$

El cuadro muestra que la diferencia es estadísticamente significativa a un nivel superior al 1 por mil.

Grupo III. — Los animales de este grupo (8) que estuvieron sometidos al mismo tratamiento que los del grupo anterior pero en los que las cifras de hematíes se restituyeron a lo normal mediante la transfusión en el momento de la invasión bacteriana, comenzaron a las pocas horas a mostrar síntomas del síndrome hemolítico y fueron muriendo en los días subsiguientes. La mortalidad el día 10º fué de 75 %.

χ^2 de mortalidad el día 10º

	Total	*Viven*	*Mueren*
Esplenectomizados sin sangría	65	14	51
Esplenectomizados sangrados y transfundidos	8	2	6

$\chi^2 = 1.60 > 0.05$

χ^2 de mortalidad el día 10º

	Total	*Viven*	*Mueren*
Esplenectomizados sangrados	26	25	1
Esplenectomizados sangrados y transfundidos	8	2	6

$\chi^2 = 14.8$

$\chi^2_{(0.001)} = 10.827$

Los cuadros muestran que los animales que han sido sangrados después de la esplenectomía y luego transfundidos tienen un porcentaje de mortalidad que no discrepa significativamente del de los solamente esplenectomizados, mientras que hay una diferencia significativa a un nivel superior al 1 por mil con los que han sido esplenectomizados y sangrados.

Grupo IV. — Se introdujo con la finalidad de comprobar "in vivo" la tolerancia de los glóbulos transfundidos, ya asegurada por pruebas de compatibilidad "in vitro". Todos los animales de este grupo (10) recuperaron sus cifras normales de glóbulos rojos a raíz de la transfusión, y los mantuvieron durante los días que duró el experimento. En ningún momento se observó en ellos hematuria macroscópica, y el estado general se mantuvo excelente.

Discusión

La observación del grupo testigo permitiría suponer que el elevado porcentaje de muertes por anemia hemolítica podría ser explicado por el trastorno nutritivo ocasionado por la anemia aguda, que alcanza alrededor de 1/3 a 1/4 de las cifras habituales de glóbulos rojos, en animales intoxicados por la infección.

Sin embargo, el grupo II muestra que si la misma pérdida se realiza hacia el exterior por la sangría, los animales no sólo no mueren sino tampoco experimentan ninguno de los síntomas de intoxicación que muestran los del grupo testigo. Y no puede discutirse que hayan sufrido la infección, primero por la visualización de los gérmenes en grandes cantidades en los frotis, y además por la lentitud de la recuperación de las cifras de hematíes, comparada con la recuperación relativamente rápida que se observa en animales normales igualmente sangrados, lo que atestigua la marcha del proceso hemolítico.

Por otra parte, los animales del grupo III han sufrido las mismas maniobras de operación, calentamiento, sangría, etc., que los del II, y la única diferencia entre los dos grupos, responsable de la gran diferencia en el porcentaje de mortalidad, es el distinto nivel de glóbulos rojos en el momento de la inundación bacteriana, con hemólisis masiva en un caso y lenta e impercentible en el otro.

Finalmente el grupo IV muestra que la producción de la hemólisis y muerte en el grupo III no puede atribuirse a fenómenos de incompatibilidad sanguínea desde que todos los animales reciben glóbulos homólogos que, no mediando la diseminación bacteriana facultada por la esplenectomía son perfectamente tolerados y conservados sin signos de destrucción masiva.

Se puede concluir entonces que la muerte en la anemia hemolítica por bartonelosis no se debe a la anemia por sí misma, sino debe ser atribuida a otras causas relacionadas con la marcha del proceso.

Una de ellas es la brusca destrucción de grandes cantidades de hematíes cuyos productos de desintegración inundan en pocas horas el organismo.

Considerando que el cuadro está producido por un germen esencialmente parasitario del glóbulo rojo, otra posible causa de la mayor supervivencia de los sangrados es la falta de sustrato en que poder multiplicarse, porque suponiendo que cada hematíe puede admitir una cantidad límite de bartonellas, cifras menores de glóbulos significan cantidades menores de gérmenes, y menor producción de toxinas y productos de desecho.

Experimentos posteriores ya en marcha permitirán dilucidar cuál de los dos mecanismos es el responsable de la elevada mortalidad.

Resumen

Si se anemiza por sangría a ratas esplenectomizadas portadoras de bartonellas, de manera que al producirse la bacteriemia las cifras de rojos estén en 3 millones o menos, la mortalidad inmediata (79.5 %) es significativamente menor que en los testigos no anemizados (3.85 %);

($p < 0.001$). Pero si en el momento de la diseminación bacteriana se transfunden glóbulos homólogos de modo que las cifras de rojos vuelvan a lo normal, anulando el efecto de la sangría, la mortalidad vuelve a ascender haciéndose igual a la de los testigos. Es posible concluir, por lo tanto, que la anemia por sí misma no es la causa de la muerte.

SUMMARY

Splenectomized rats carriers of Bartonella muris, bled for three days after splenectomy in order to lower the red cell levels until about three millions at the onset of the bacteriemia, die significantly less (3.85 % than nonbled splenectomized controls (79.5 %, $p < 0.001$).

Homologous blood transfusion performed in the third day of operation when bacteriemia begins to appear, returning erythrocytic levels to normal values, increases the percentage of death until approximately that observed in controls.

It is concluded, therefore, that anemia by itself is not the cause of death in bartonelosis.

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PROCEEDINGS OF THE ARGENTINE SOCIETY OF BIOLOGY

Buenos Aires, July 5th, 1956

Effect of corticoadrenal hormones on the adrenaline and noradrenaline content of the toad adrenal. C. E. RAPELA AND M. F. GORDON. *Instituto de Biología y Medicina Experimental, Costa Rica 4185, Buenos Aires, Argentina.*

Hypophysectomy produces a diminution of adrenaline in the adrenal gland of the toad. In the hypophysectomized animal, hydrocortisone injections increase the adrenaline and noradrenaline; adrenocorticotrophin increases almost exclusively, the adrenaline. Both hydrocortisone and adrenocorticotrophin do not modify the catechols of the adrenals in normal toads. Desoxycorticosterone increases the proportion of adrenaline in the adrenal gland of normal male toads.

Effect of adrenaline, noradrenaline and acetylcholine on the oviduct of the turtle "Chrysemys d'Orbignyi". M. MARQUES. *Instituto de Fisiología Experimental, Facultad de Medicina, Porto Alegre, R. G. do Sul, Brasil.*

Diabetogenic action of pituitary hormones on adrenalectomized hypophysectomized dogs. B. A. HOUSSAY AND J. C. PENHOS. *Instituto de Biología y Medicina Experimental, Costa Rica 4185, Buenos Aires, Argentina.*

Pituitary hormones: somatotrophin and prolactin had a diabetogenic effect in dogs deprived of hypophysis and adrenals and with pancreas surgically reduced to 15-18 % of its mass. These facts demonstrate that the hypophysis and the adrenals are not essential for the production of the diabetogenic effect of these hormones. Adrenocorticotrophin had no action on the glycemia of these adrenalectomized dogs. The animals showed moderate symptoms of asthenia which in some cases were marked. The animals recovered rapidly when injected with cortisone or hydrocortisone.

Hydrocortisone, at a doses of 3 mg/kg/day, produced in a short time, a diabetic hyperglycemia.

The dogs with reduced pancreas and deprived of one adrenal and the hypophysis developed a marked adiposity.